Medical Mycology September 2006, **44**, 485–492

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Role of birds of prey as carriers and spreaders of *Cryptococcus neoformans* and other zoonotic yeasts

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In the last 20 years, cases of human cryptococcosis, have increased in immunocompromised patients. In several instances, the cases have been associated with the exposure of the patients to bird droppings. In order to investigate birds of prey as potential carriers and spreaders of *Cryptococcus neoformans* and other yeasts of importance in human infections, 182 swab samples were collected from the cloacae of several species of birds of prey (Group I) and 32 faecal samples from aviaries in which the birds were housed (Group II). Samples were also taken from digestive tract of 60 dead birds (Group III). A total of 454 samples were cultured from which 215 colonies of yeastlike fungi were recovered and identified. *Cryptococcus neoformans* var. *grubii* was isolated from three cloacae samples (4.8%) collected from *Falco tinnunculus* and from one sample (3.1%) obtained from *Buteo buteo*, as well as from samples collected at the aviaries in which these birds were kept. Overall, 18 samples (9.9%) from Group I, 13 (40.6%) from Group II, 12 crops (20%), three proventriculi (5%) and 12 cloacae (20%) from Group III yielded positive cultures for yeasts. The results indicate that birds of prey and in particular, *F. tinnunculus* and *B. buteo*, may act as carriers and spreaders of *C. neoformans* and other zoonotic yeasts.

Keywords Birds of prey, *Cryptococcus neoformans*, *Candida* spp., droppings, yeasts

Introduction

In the past 20 years, yeasts (mainly *Cryptococcus neoformans* and *Candida* spp.) have been reported as among the most important causes of human infections, mainly in immunocompromised and cancer patients [1–4]. Only one species of *C. neoformans* with two varieties and five serotypes was previously recognized [5–8]. However, following the proposals of Franzot *et al*. [5] and Kwon-Chung *et al*. [9], this single species was divided into two, (i.e., *C. neoformans* and *Cryptococcus gattii*) and five different serotypes (i.e., *C. neoformans* var. *neoformans* (serotypes D and AD), *C. neoformans* var. *grubii* (serotype A) and *C. gattii* (serotypes B and C)) are generally accepted. *Cryptococcus gattii* is geographically restricted to tropical and subtropical areas, but it has also been recently reported in Southern Italy [10]. *C. gattii* is associated with trees of the genus *Eucalyptus* [11–14] and usually causes infection in immunocompetent individuals [15]. By contrast, *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* are commonly found throughout the world in association with soil and avian excreta [11,16,17] and are generally responsible for cryptococcosis in immunocompromised individuals [18]. The presence of *C. neoformans* has also been reported in droppings of several birds such as psittacides [19,20], passeriformes [19,21], columbiformes [19] and falconiformes [22]. Reports of human cryptococcosis have increased over the past few years in association with a rise in immunodeficiency syndromes and have been described in several patients exposed to bird droppings

Received 28 December 2006; Accepted 1 April 2006
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DOI: 10.1080/13693780600735452

[20,23–29]. The clinical symptoms and prognosis in these individuals vary in relationship to their immunological status [4].

Birds of prey have a high potential to disseminate zoonotic agents due to the vast distances they cover and because they are increasingly kept in raptor centres for conservation or rehabilitation purposes. No data are available on the role of these birds as carriers of pathogenic yeasts. Furthermore, studies on yeasts isolated directly from the lower digestive tract of birds are scant and, when available, are limited to feral pigeons [30–32] and migratory birds [33].

Thus, the aim of the present work was to study the occurrence of *C. neoformans* and other yeasts in the cloacae of different species of birds of prey and in the habitat where they were sheltered in order to evaluate the role of birds as carriers and spreaders of zoonotic yeasts.

Materials and methods

Sampling procedures

From October 2001 to May 2003, samples were collected from hospitalized birds (Group I), their respective aviaries (Group II) and from dead animals (Group III), all from the Apulia Regional Fauna Observatory, province of Bari, Southern Italy (ARFO).

In Group I 182 birds of prey belonging to different families (Falconidae, Accipitridae, Strigidae and Titonidae) were analyzed. The animals were housed at ARFO because they were retrieved unable to fly. Animals that were in particularly poor condition were admitted to primary care aviaries, treated, then transferred to secondary care aviaries. Samples were collected upon birds' recovery (i.e., before the birds went to the aviaries) from their cloacae using sterile cotton swabs. The number and species of hospitalized birds sampled are reported in Table 1.

In Group II 32 aviaries (i.e., 12 primary care and 20 secondary care aviaries) were analyzed (Table 1). Only one bird was present in each primary care aviary, while many birds belonging to the same species were in each of the secondary care aviaries. The droppings of the hospitalized birds were collected from the aviaries 3 days post-admission. One gram of faeces was suspended in 9 ml sterile saline solution (NaCl 0.9%) containing 1000 μg/ml streptomycin and 500 UI penicillin/ml.

Group III included all the birds that died while housed in ARFO. Dead birds were delivered within 24h to the mycological laboratory of the Faculty of Veterinary Medicine, University of Bari, and submitted to necropsy. Sixty birds of different species were analyzed (Table 2). The birds were examined and their ages were determined as young or adult based on plumage (Table 2) [34]. For each sample, the digestive tract was isolated and dissected into four parts (i.e., crop, proventriculus, ventriculus and cloacae) and samples were collected from each using sterile cotton swabs.

Table 1 Number of hospitalized birds (Group I) and number of aviaries from which each species was taken (Group II)

Species of birds	Hospitalized birds	Aviaries
Kestrel – *Falco tinnunculus*	63	3
Hobby – *Falco subbuteo*	3	1
Lesser Kestrel – *Falco neumanni*	19	3
Peregrine – *Falco peregrinus*	2	1
Marsh Harrier – *Circus aeruginosus*	8	2
European Honey Buzzard – *Pernis apivorus*	2	1
Montagu's Harrier – *Circus pygargus*	8	1
Buzzard – *Buteo buteo*	32	3
Black Kite – *Milvus migrans*	2	2
Eurasian Sparrowhawk – *Accipiter nisus*	2	1
Short-toed Eagle – *Circaetus gallicus*	2	1
Scops Owl – *Otus scops*	5	1
Eurasian Pygmy-Owl – *Glaucidium passerinum*	10	5
Short-eared Owl – *Asio flammeus*	5	2
Long-eared Owl – *Asio otus*	14	2
Eurasian Eagle-Owl – *Bubo bubo*	1	1
Tawny Owl – *Strix aluco*	1	1
Barn Owl – *Tyto alba*	3	1
Total	182	32

Mycological culture and identification procedures

Samples (i.e., swabs from Groups I and III, or 0.1ml of droppings solution from Group II) were cultured onto Sabouraud dextrose agar with chloramphenicol-0.5 gr/l (BioLife®) (SAB agar) added by biphenyl 0.1%, incubated at 30°C for 7 days and observed daily. For each positive sample, colonies were examined microscopically after Gram staining to avoid bacterial contamination. Since no more than 200 non-confluent colonies of yeasts per plate can be clearly identified by visual inspection, the maximum number of colonies counted per plate was 200 colony forming units (CFU). Four colonies were sub-cultured in SAB agar slants for identification at species level. The identification was based on microscopic morphology, urea hydrolysis and sugar assimilation [35]. In particular sugar assimilation was tested by ID32C and Vitek System (bioMérieux®). The isolates of *C. neoformans* were also identified by observing dark colonies on Staib medium [36] and by

Table 2 Number of dead birds (Group III) examined, grouped according to sex and age

Species of birds	Males	Females	Young	Adult	Total
Kestrel *Falco tinnunculus*	11	10	6	15	21
Lesser Kestrel *Falco neumanni*	2	4	3	3	6
European Honey Buzzard *Pernis apivorus*	1	2	2	1	3
Montagu's Harrier *Circus pygargus*	3	6	4	5	9
Buzzard *Buteo buteo*	11	7	4	14	18
Long-eared Owl *Asio otus*	1	2	0	3	3
Total	29	31	19	41	60

the presence of capsules stained by India Ink. *Candida albicans* was detected by germ tube production [35]. To determine the varieties and serotypes of *C. neoformans* strains, the isolates were inoculated onto canavanine glycine-bromothymol blue agar medium (CGB) for 48 h [13] and then serotyped using monoclonal antibodies specific for capsular polysaccharides (Crypto Check-Iatron Laboratories, Tokyo, Japan).

The differences in mean CFU in samples from different groups of animals were statistically analysed

Table 3 Number and percentage (in brackets) of samples that were positive for yeasts. Mean colony forming unit (CFU)/sample and Standard deviation (sd) are also reported

Species of birds	Group I	Group II		Group III							
	Pos/tot (%)	Means CFU (sd)	Pos/tot (%)	Means CFU (sd)	Crops		Proventriculus		Ventriculus		Cloacae	
					Pos/tot (%)	Means CFU (sd)	Pos/tot (%)	Means CFU (sd)	Pos/tot (%)	Means CFU (sd)	Pos/tot (%)	Means CFU (sd)
Kestrel *Falco tinnunculus*	8/63 (12.7)	53 (90.8)	2/3 (66.6)	200 (0.00)	6/21 (28.6)	17.50 (5.39)	3/21 (14.28)	10 (5.29)	0/21		9/21 (42.8)	15.89 (7.88)
Hobby *Falco subbuteo*	1/3 (33.0)	18	1/1 (100)	200	nd	–	nd	–	nd	–	nd	–
Lesser Kestrel *Falco neumanni*	4/19 (21.0)	3 (2.45)	2/3 (66.6)	125 (106)	3/6 (50)	10 (5)	0/6		0/6		0/6	
Peregrine *Falco peregrinus*	0/2 (0.0)	–	1/1 (100)	70	nd	–	nd	–	nd	–	nd	–
Buzzard *Buteo buteo*	3/32 (9.3)	7 (6.24)	2/3 (66.6)	200 (0.00)	3/18 (16.6)	79.67 (17.5)	0/18		0/18		3/18 (16.6)	51 (20.52)
Black Kite *Milvus migrans*	0/2 (0.0)	–	1/2 (50)	200	nd	–	nd	–	nd	–	nd	–
Short-toed Eagle *Circaetus gallicus*	0/2 (0.0)	–	1/1 (100)	20	nd	–	nd	–	nd	–	nd	–
Scops Owl *Otus scops*	1/5 (0.0)	12	1/1 (100)	200	nd	–	nd	–	nd	–	nd	–
Eurasian Pygmy-Owl *Glaucidium passerinum*	1/10 (10%)	9	3/5 (60)	158.67 (71.59)	nd	–	nd	–	nd	–	nd	
Total	18/182 (9.9)	27.56[a] (62.94)	14/32 (43.7)	155.08[abcd] (71.26)	12/60 (20)	31.17[b] (30.64)	3/60 (5)	10 (5.29)[c]	0/60	Neg	12/60 (20)	24.67[d] (19.34)

Nd: not done. [a-d] Student *t*- test: Statistically significant differences ($P < 0.05$) where marked with the same letters.

Table 4 Number and percentage (in brackets) of hospitalized birds (Group I) that were positive for yeasts. Birds were grouped according to the yeasts isolated. Number of isolates (I) is also indicated

Species of yeasts	Kestrel *Falco tinnunculus*		Hobby *Falco subbuteo*		Lesser Kestrel *Falco neumanni*		Buzzard *Buteo buteo*		Scops Owl *Otus scops*		Eurasian Pygmy-Owl *Glaucidium passerinum*		Total	
	Pos/Tot (%)	I	Pos/Tot (%)	I	Pos/Tot (%)	I	Pos/Tot (%)	I	Pos/Tot (%)	I	Pos/Tot (%)	I	Pos/Tot (%)	I (%)
Cryptococcus neoformans	3/63 (4.8)	12	–	–	–	–	1/32 (3.1)	4	–	–	–	–	42.2	16/51 (31.4)
Cryptococcus laurentii			1/3 (33.3)	4									1 (0.5)	4/51 (7.8)
Candida albicans	–	–	–	–	–	–	–	–	1/5 (20)	4	–	–	1 (0.5)	4/51 (7.8)
Candida incospicua	1/63 (1.6)	4	–	–	–	–	–	–	–	–	–	–	1 (0.5)	4/51 (7.8)
Candida pelliculosa	1/63 (1.6)	1	–	–	–	–	–	–	–	–	–	–	1 (0.5)	1/51 (1.9)
Candida tropicalis	–	–	–	–	–	–	–	–	–	–	1/10 (10)	4	1 (0.5)	4/51 (7.8)
Candida famata	–	–	–	–	–	–	1/32 (3.1)	2	–	–	–	–	1 (0.5)	2/51 (3.9)
Rhodotorula rubra	3/63 (4.8)	4	–	–	4/19 (21)	10	1/32 (3.1)	2	–	–	–	–	8 (4.4)	16/51 (31.4)
Total	8/63 (12.7)	21	1 (33.3)	4	4/19 (21)	10	3 (9.4)	8	1/5 (20)	4	1 (10)	4	18/182 (9.9)	51

using the Student t-test. A value of $p \leq 0.05$ was considered to be statistically significant.

Results

The number and percentage of yeast-positive samples, along with population size in Groups I, II and III, are reported in Table 3. The prevalence of different yeast for each bird species in Group I, and the number of isolates recovered from each are summarized in Table 4. A total of 56 isolates belonging to seven species of yeasts were identified in Group II (Table 5). Yeast species isolated from the aviaries were also retrieved from the cloacae of the animals housed in the aviaries with the exception of *Cryptococcus albidus* and *Cryptococcus albidus* (Table 4 and 5). The prevalence of different yeast species and the number of isolates from different anatomical sites of birds in Group III are reported in Table 6. *Candida albicans*, *Candida famata* and *Candida guilliermondii* were retrieved from both crops and cloacae, *Rhodotorula rubra* from ventriculi and cloacae and *Candida parapsilosis* from only crops (Table 6). In addition, *Candida incospicua, Candida pelliculosa, C. famata, Candida parapsilosis* and *C. guilliermondii* were isolated only from the cloacae of hospitalized birds and/or from the digestive tract of dead birds, but not from the environment. *Cryptococcus neoformans* was isolated from four birds (2.2%) in Group I and from the samples collected at four of the aviaries housing the same birds (Table 4 and 5), but never from any anatomical location (Group III – Table 6). All the isolates of *C. neoformans* were identified as *C. neoformans* var. *grubii*.

Discussion

The results of the present work indicate that birds of prey may act as carriers and spreaders in the environment of *C. neoformans* and other potentially zoonotic yeasts as demonstrated by the detection of *C. neoformans* var. *grubii* from the cloacae of *F. tinnunculus* and *B. buteo*, as well as from the aviaries in which these species were kept. The isolation of *C. neoformans* var. *grubii* from the aviary in which *Milvus migrans* was housed, but not from their cloacae, might be due to the fact that these birds had been fed contaminated food, e.g., chicken wings, necks and internal organs, after hospitalization. *Milvus migrans* droppings may also have been contaminated by *C. neoformans* from the presence of the yeast in environmental sources such as the soil and air. The fungus can reach a very small size in external habitats and can easily be disseminated by the wind [37]. *C. neoformans* was never isolated from the digestive tract of necropsied animals. Interestingly the isolation of only *C. neoformans* var. *grubii* confirms

Table 5 Number and percentage (in brackets) of aviaries (Group II) that were positive for yeasts. Aviaries were grouped according to the species of yeasts isolated. The number of isolates (I) is also indicated

Species of yeasts	Kestrel *Falco tinnunculus*		Hobby *Falco subbuteo*		Lesser Kestrel *Falco neumanni*		Peregrine *Falco peregrinus*		Buzzard *Buteo buteo*		Black Kite *Milvus migrans*		Short-toed Eagle *Circaetus gallicus*		Scops Owl *Otus scops*		Eurasian Pygmy-Owl *Glaucidium passerinum*		TOT	
	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I	Pos/tot (%)	I (%)
Cryptococcus neoformans	2/3 (66.6)	8							1/3 (33.3)	4	1/2 (50)	4							4/32 (12.5)	16/56 (28.6)
Cryptococcus laurentii			1/3 (33.3)	4															1/32 (3.1)	4/56 (7.1)
Cryptococcus albidus					1/10 (10)	4													1/32 (3.1)	4/56 (7.1)
Candida albicans															1/5 (20)	4	1/10 (10)	4	2/32 (6.2)	8/56 (14.3)
Candida tropicalis																	1/10 (10)	4	1/32 (7.7)	4/56 (7.1)
Trichosporon cutaneum																	1/10 (10)	4	1/32 (3.1)	4/56 (7.1)
Rhodotorula rubra					1/10 (10)	4	1/2 (50)	4	1/3	4			1/2 (50)	4					4/32 (12.5)	16/56 (28.6)
Total	2/3 (66.6)	8	1/3 (33.3)	4	2/10 (20)	8	1/2 (50)	4	2/3 (66.6)	8	1/2	4	½ (50)	4	1/5 (20)	4	3/10 (30)	12	14/32 (43.7)	56

Table 6 Number and percentage of dead birds (Group III) that were positive for yeasts in the digestive tract. The positive samples were grouped according to the yeasts isolated and the number of isolates from crop (C), proventriculus (P), ventriculus (V) and cloaca (Cl) is reported

Yeasts Isolated	*Falco timunculus*						*Buteo buteo*						*Falco neumanni*						Total					
	Pos/tot (%)	Isolates					Pos/tot	Isolates					Pos/tot	Isolates					Pos/tot	Isolates				
		Tot	C	P	V	Cl		Tot	C	P	V	Cl		Tot	C	P	V	Cl		Tot (%)	C	P	V	Cl
Candida albicans	3/21 (14.3)	24	12	–	–	12	3/18 (16.6)	24	12	–	–	12							6/60 (10)	48/108 (44.4)	24			24
Candida parapsilosis	–	–		–	–		–			–	–		3/6* (50)	6	6*	–	–		3/60* (5)	6 (5.5)	6			
Candida famata	3/21+ (14.3)	6	–	–	–	6	–			–	–		3/6* (50)	6	6*	–	–		6/60*+ (10)	12/108 (11.1)	6			6
Candida guilliermondii	3/21 (14.3)	24	12	–	–	12	–			–	–					–	–		3/60+ (5)	24/108 (22.2)	12			12
Rhodotorula rubra	3/21* (14.3)	18	–	12	–	6	–			–	–					–	–		3/60 (5)	18/108 (16.6)		12		6
Total	9/21 (42.8)	72	24	12		36	3/18 (16.6)	24	12	–	–	12	3/6* (50)	12	12	–	–		15/60 (25)	108	48	12		48

+*C. famata* and *R. rubra* were isolated from cloacae of the same three birds.
**C. parapsilosis* and *C. famata* were isolated from crops of the same three birds.

previous findings of the same variety in birds from Southern Italy [17,38].

Overall, the prevalence of yeasts isolated from cloacae (Group I = 9.9%) was lower than the rates reported in the literature for migratory birds (15.7%) [33] and pigeons (30%) [31,32], and it varied among bird species. In this respect the number of isolated yeasts may be influenced by the diet, behaviour and ecology of the examined birds. Nevertheless the statistically higher number of yeasts found in the aviaries, compared to the cloacae or digestive tracts, indicates that excreta are an enriched medium that permit the growth of yeasts. *Candida albicans* and *R. rubra* were the most common species isolated from the cloacae of hospitalized birds, the digestive tract of dead birds and from the aviaries, in accord with previous studies of pigeon droppings [39] and the cloacae of migratory birds [33]. The finding of other yeast species (i.e., *C. incospicua, C. pelliculosa, C. famata, C. parapsilosis* and *C. guilliermondii*) only in the cloacae of hospitalized birds and in the digestive tract of dead birds, but not in the environment, might indicate that they belong to the normal flora of the digestive tract of birds of prey. In particular, the distribution of yeasts (Table 6) might be influenced by the physiological characteristics of different digestive tracts (i.e., pH, temperature) [35,40]. Yeasts isolated in this survey have been gaining greater importance in medical mycology over the past two decades [41,42]. In particular cryptococcosis and candidosis have been reported with increased frequency, especially in immunocompromised patients (e.g., individuals undergoing solid-organ transplantation, neoplastic diseases, immunosuppressive therapy and AIDS) [1–3,43,44].

The results of this work demonstrate that birds of prey may harbour in their cloacae different species of potentially pathogenic yeasts and may be capable of disseminating these fungi in the environment. In particular, *F. tinnunculus* and *B. buteo* harboured the highest number of yeasts (mainly *C. neoformans* and *C. albicans*). Furthermore, presence of the two above-mentioned bird species, which rest during their flight in neighbouring towns or in raptor centres, makes these birds a focus of interest as possible carriers and spreaders of pathogenic fungi. These results are particularly interesting also from the sociological point of view, since these sites are frequented by children and the elderly, as well as immunocompromised patients who are considered at high risk for contracting opportunistic diseases.

Acknowledgements

The authors are grateful to the Editors and two anonymous reviewers who provided helpful comments on the manuscript.

References

1 Antinori S, Galimberti L, Magni C, *et al*. *Cryptococcus neoformans* infection in a cohort of Italian AIDS patients: natural history, early prognostic parameters, and autopsy findings. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 711–717.
2 Hazen KC. New and emerging yeast pathogens. *Clin Microbiol Rev* 1995; **8**: 462–478.
3 Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS-100 Years after discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 1995; **8**: 515–548.
4 Montagna MT, Tortorano AM, Fiore L, Viviani MA, Barbuti S. *Cryptococcus neoformans* var. *gattii* en Italie. Note I. Premier cas autochtone de mëningite à sérotype B chez un sujet VIH positif. *J Mycol Med* 1997; **7**: 9220–9225.
5 Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol* 1999; **37**: 838–840.
6 Evans EE. The antigenic composition of *Cryptococcus neoformans*. I. A serologic classification by means of the capsular and agglutination reactions. *J Immunol* 1950; **64**: 423–430.
7 Vanbreuseghem R, Takashio M. An atypical strain of *Cryptococcus neoformans* (Sanfelice) Vuillemin, 1894. II. *Cryptococcus neoformans* var. *gattii* var. nov. *Ann Soc Belg Med Trop* 1970; **50**: 695–702.
8 Wilson DE, Bennett JE, Bailey JW. Serologic grouping of *Cryptococcus neoformans*. *Proc Soc Exp Biol Med* 1968; **127**: 820–823.
9 Kwon-Chung KJ, Boekhout T, Fell JW, Diaz M. Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (*Basidiomycota, Hymenomycetes, Tremellomycetidae*). *Taxon* 2002; **51**: 804–806.
10 Montagna MT. A note on the isolation of *Cryptococcus neoformans* serotype A MATa strain from the Italian environment. *Med Mycol* 2002; **40**: 593–595.
11 Ellis DH, Pfeiffer TJ. Ecology, life cycle, and infectious propagule of *Cryptococcus neoformans*. *Lancet* 1990; **13**: 923–925.
12 Kwon-Chung KJ, Bennett JE. High prevalence of *Cryptococcus neoformans* var. *gattii* in tropical and subtropical regions. *Zentralbl Bakteriol Mikrobiol Hyg [A]* 1984; **257**: 213–218.
13 Min KH, Kwon-Chung KJ. The biochemical basis for the distinction between the two *Cryptococcus neoformans* varieties with CGB medium. *Zentralbl Bakteriol Mikrobiol Hyg [A]* 1986; **261**: 471–480.
14 Pfeiffer TJ, Ellis DH. Environmental isolation of *Cryptococcus neoformans* var. *gattii* from *Eucalyptus tereticornis*. *J Med Vet Mycol* 1992; **30**: 407–408.
15 Chen S, Sorrell T, Nimmo G, *et al*. Epidemiology and host- and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Australasian Cryptococcal Study Group. *Clin Infect Dis* 2000; **31**: 499–508.
16 Ruiz A, Fromtling RA, Bulmer GS. Distribution of *Cryptococcus neoformans* in a natural site. *Infect Immun* 1981; **31**: 560–563.
17 Montagna MT, Mele MS, De Donno A, Marcuccio C, Pulito A. Criptococcosi e AIDS. Nota 1: Indagini sulla diffusione di *Cryptococcus neoformans* nella città di Bari e di Lecce. *Rivista Italiana di Igiene* 1996; **56**: 69–77.
18 Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin Infect Dis* 1995; **21**: 28–34.
19 Bauwens L, Swinne D, De Vroey C, De Meurichy W. Isolation of *Cryptococcus neoformans* var. *neoformans* in the aviaries of the Antwerp Zoological Gardens. *Mykosen* 1986; **29**: 291–294.
20 Nosanchuk JD, Shoham S, Fries BC, *et al*. Evidence of zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised patient. *Ann Intern Med* 2000; **132**: 205–208.
21 Criseo GM, Bolignano MS, De Leo F, Staib F. Evidence of canary droppings as an important reservoir of *Cryptococcus neoformans*. *Zentralbl Bakteriol* 1995; **282**: 244–254.
22 Caicedo LD, Alvarez MI, Delgado M, Cardenas A. *Cryptococcus neoformans* in bird excreta in the city zoo of Cali, Colombia. *Mycopathologia* 1999; **147**: 121–124.
23 Currie BP, Freundlich LF, Casadevall A. Restriction fragment length polymorphism analysis of *Cryptococcus neoformans* isolates from environmental (pigeon excreta) and clinical sources in New York City. *J Clin Microbiol* 1994; **32**: 1188–1192.
24 Fessel WJ. Cryptococcal meningitis after unusual exposures to birds. *N Engl J Med* 1993; **328**: 1354–1355.
25 Garcia-Hermoso D, Mathoulin-Pelissier S, Couprie B, *et al*. DNA typing suggests pigeon droppings as a source of pathogenic *Cryptococcus neoformans* serotype D. *J Clin Microbiol* 1997; **35**: 2683–2685.
26 Haag-Wackelnagel D. Street pigeons in Basel. *Nature* 1993; **361**: 200.
27 Kumlin U, Olsen B, Granlund M, Elmqvist LG, Tarnvik A. Cryptococcosis and starling nests. *Lancet* 1998; **351**: 1181.
28 Yamamoto Y, Kohno S, Koga H, *et al*. Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. *J Clin Microbiol* 1995; **33**: 3328–3332.
29 Lagrou K, Van Eldere J, Keuleers S, *et al*. Zoonotic transmission of *Cryptococcus neoformans* from a magpie to an immunocompetent patient. *J Intern Med* 2005; **257**: 385–388.
30 Guigen C, Boisseau-Llebreuil MT, Couprie CB. Flore fongique du tube digestif isolée de pigeons de ville à Bordeaux. *Bull Soc Franc Mycol Méd* 1986; **1**: 151–154.
31 Mattsson R, Haeming PD, Olsen B. Feral pigeons as carriers of *Cryptococcus laurentii, Cryptococcus uniguttulatus* and *Debaryomyces hansenii*. *Med Mycol* 1999; **37**: 367–369.
32 Ramirez R, Robertstad GW, Hutchinson LR, Chavez J. Mycotic flora in the lower digestive tract of feral pigeons (*Columba livia*) in the El Paso, Texas area. *J Wildl Dis* 1976; **12**: 83–85.
33 Cafarchia C, Camarda A, Romito D, *et al*. Occurrence of yeasts in cloacae of migratory birds. *Mycopathologia* 2006; **161**: 229–234.
34 Clark WS. *A field guide to the raptors of Europe, Middle East and North Africa*. Oxford: Oxford University Press, 1999.
35 Barnett JA, Payne RW, Yarrow D. *Yeasts: Characteristics and Identification*, 3rd ed. Edinburgh: Cambridge University Press, 2000.
36 Staib F. New concepts in the occurrence and identification of *Cryptococcus neoformans*. *Mycopathol Mycol Appl* 1963; **19**: 143–145.
37 Chee HY, Lee KB. Isolation of *Cryptococcus neoformans* var. *grubii* (serotype A) from pigeon droppings in Seoul, Korea. *J Microbiol* 2005; **43**: 469–472.
38 Criseo G, Gallo M. Serotyping of *Cryptococcus neoformans* isolates from environmental and clinical sources in extreme

southern Italy (Calabria and Sicily, central Mediterranean area). *Mycoses* 1997; **40**: 95–100.
39 Gallo MG, Cabeli P, Vidotto V. Presence of pathogenic yeasts in the feces of the semi-domesticated pigeon (*Columba livia*, Gmelin 1789, urban type) from the city of Turin. *Parassitologia* 1989; **31**: 207–212.
40 Martinez LR, Garcia-Rivera J, Casadevall A. *Cryptococcus neoformans* var. *neoformans* (serotype D) strains are more susceptible to heat than *C. neoformans* var. *grubii* (serotype A) strains. *J Clin Microbiol* 2001; **39**: 3365–3367.
41 Hajjeh RA, Sofair AN, Harrison LH, *et al*. Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. *J Clin Microbiol* 2004; **42**: 1519–1527.
42 Pfaller MA, Diekema DJ; International Fungal Surveillance Participant Group. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. *Clin Microbiol Infect* 2004; 10: 11–23.
43 Anaissie E, Bodey GP, Kantarjian H, *et al*. New spectrum of fungal infection in patients with cancer. *Rev Infect Dis* 1989; **11**: 369–378.
44 Samonis G, Bafaloukos D. Fungal infections in cancer patients: an escalating problem. *In vivo* 1992; **6**: 183–194.